Synchrotron Infrared Microspectroscopy of Programmed Cell Death

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A spectrum of a necrotic cell showed that all the infrared bands are much broader than those observed with viable cells. The most striking difference was the appearance of a narrow band that was assigned to carbonyl ester groups. In the future, we hope to extend this infrared microspectroscopic technique to monitoring changes in the intracellular localization of chemical compounds occurring inside individual cells during various biological events such as mitosis, drug uptake, receptor activation, endocytosis, and apoptosis.

Currently, we are developing methods to examine cells suspended in their media. To this point, cells have been deposited onto a BaF_2 disk with a cytospin, removing excess liquid and essentially suspending all activity but not killing the cells. We have been successful transferring cells in media made from a 50:50 mixture of D_2O and H_2O and placing the solution between two BaF_2 disks. Using this technique, we are investigating the process of apoptosis using an anti-FAS mouse antibody system. Results thus far demonstrate a dramatic split of the Amide I band, where a lower frequency component increases as apoptosis progresses. The growth of this band near 1633 cm^{-1} is likely due to protein degradation and aggregation in the cell. In addition, we see the growth in a carbonyl ester band near 1735 cm^{-1}, also demonstrating protein degradation and oxidation in apoptosis.